THE AGENT FOR ASSAYING ANALYTE OF PATIENT BY ENZYME

Technical field

This invention involves a reagent for enzymatic determination of an analyte concentration in a patient, especially involves the reagents which measures the degree of oxidation of reduced coenzyme which quantity corresponds directly to the concentration of analyte present in the sample.

Technology background

In clinic, analytes that can be measured by determing the degree of oxidation of β-NADH include aspartate aminotransferase, alanine aminotransferase, ammonia, urea, lactate dehydrogenase, carbon dioxide and α-hydroxyl butyric acid dehydrogenase.

Aspartate aminotransferase is widely distributed in human body espeacialy higher in heart, liver, kidney, red blood cells and skeletal muscle . Increases of levels of aspartate aminotransferase in serum are found in tissue destruction such as myocardial infarction, liver cell destruction, hepatitis, hepatocirrhosis, malnutrition.

When the activity of aspartate aminotransferase(AST) is determined AST in the serum catalyses amino transformation from α-ketoglutarate to L-aspartate to form L- glutamic acid and oxaloacetate. In the presence of reduced coenzyme I (β-NADH) and malate dehydrogenase (MDH), oxaloacetate is converted to malate. This is accompanied by the oxidation of the coenzyme nicotinamide adenine dinucleotide (β-NADH toβ-NAD^+) which can lower the absorbance at 340nm. Thus the reaction sequence is commonly as follows:

$$\text{L-aspartate} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{AST}} \text{oxaloacetate} + \text{glutamate}$$

$$\text{oxaloacetate} + \beta\text{-NADH} \xrightleftharpoons{\text{MDH}} \text{malate} + \beta\text{-}NAD^+$$

Lactate dehydrogenase that exists in serum can convert intrinsic pyruvate to lactic acid and oxidize β-NADH, as a result it interferes with determination. High levels of lactate dehydrogenase can quickly eliminated this side reaction in the lag phase. The reaction is as follows:

$$\text{pyruvate} + \beta\text{-NADH} \xrightleftharpoons{\text{LDH}} \text{L- lactate} + \beta\text{-}NAD^+$$

Alanine aminotransferase (ALT) is existed in high concentration in the liver but low levels in heart, kidney, lung and skeletal muscle. Usually increasement in the level of ALT in the serum is concerned with some liver diseases including hepatocirrhosis, liver cancer, hepatitis,obstructive and icterus.

When the activity of alanine aminotransferase (ALT) is determined ALT in the serum catalyse amino transformation from L-alanine to α-oxoglutarate to form L-glutamate and pyruvate. In the presence of reduced coenzyme I (β-NADH) and

lactate dehydrogenase (LDH), pyruvate is converted to L-lactate. This is accompanied by the oxidation of the coenzyme nicotinamide adenine dinucleotide (β-NADH toβ-NAD^+) which can lower absorbance at 340nm. Thus the reaction sequence is as follows:

$$\text{L-alanine} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{ALT}} \text{pyruvate} + \text{L- glutamate}$$

$$\text{pyruvate} + \beta\text{-NADH} \xrightleftharpoons{\text{LDH}} \text{L- lactate} + \beta\text{-}NAD^+$$

The interference by intrinsic pyruvate in serum can be eliminated through adding excessive lactate dehydrogenase. The reaction is as follows:

$$\text{pyruvate} + \beta\text{-NADH} \xrightleftharpoons{\text{LDH}} \text{L- lactate} + \beta\text{-}NAD^+$$

Urea is the major nitrogen-containing metabolic product from protein catabolism, being formed in the liver and excreted through the kidneys. Elevated levels of urea in serum may be a consequence of impaired kidney function and urethra block. Hence the level of urea in blood is an importnt sign of kidney function.

When the concentration of urea is determined urea decomposes to ammonia and carbon dioxide in catalysis by urease. The ammonia and α-ketoglutarate is converted to glutamate in the presence ofβ-NADH and glutamate dehydrogenase (GLDH). Simultaneously β-NADH is oxidized to β-NAD^+ which can lower the absorbance at 340 nm. So the concentration of urea can be determinated by spectrophotometric method. The reactions are as follows:

$$\text{Urea} + H_2O \xrightleftharpoons{\text{urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{2-ketoglutarate} + \beta\text{-NADH} \xrightleftharpoons{\text{GLDH}} \text{glutamate} + \beta\text{-}NAD^+$$

The interference by intrinsic ammonium of serum can be quickiy eliminated in the delay lag. The reaction is as follows:

$$NH_3 \text{ (intrinsic)} + \text{2-ketoglutarate} + \beta\text{-NADH} \xrightleftharpoons{\text{GLDH}} \text{glutamate} + \beta\text{-}NAD^+$$

In order to stabilize the assay reagens for long including AST,ALT and UREA in a single vial format, it is important to resolve the stability of β-NADH and tool enzymes. Since various enzymes are precisely constructed protein which show poor stability, many factors including temperature, pH, ion strength, impurities, metal ions and microorganisms all can affect their activity. To improve the enzyme stability in aqueous solution, it is feasible to improve the surroundings of the enzymes including addition of preservatives and stabilizers and so on . Tool enzymes should be selected from enzymes which show high thermostability, less impurities, and a good stability in the pH range of determination. The quantity of tool enzymes should be appropriate in order to ensure the exactness of the assay result and the tool enzyme can stabilize for a long time.

The difficulty to assure reagent stability mainly lies in the stability of

β-NADH which is the common indicator for the assay reagents: AST,ALT and UREA. In order to guarantee the proper linearity in the assay, β-NADH in the reagent should maintain in a suitable concentration, namely the absorbance at 340nm can not be lower than 1.0A. Butβ-NADH in aqueous solution at pH<8.6 is unstable and can spontaneously be oxidized toβ-NAD^+, and can be catalized toβ-NAD^+ by other enzymes in the solution .

In order to increase the stability of β-NADH, some people had made massive research works in 1970's. They utilized general physical methods, such as freezing and drying the reagent into powder, or increased the NADH stability with some anhydrous organic solvents. In 1977, Modrovich (US 4,394,449) stated that Glucose-6-Phosphate dehydrogenase(G-6-PDH) /Glucose-6-Phosphate(G-6-P) pair can revert the productβ-NAD^+ to β-NADH, and stabilizeβ-NADH in the reagent The reaction is as folows:

$$\beta\text{-NAD}^+ + \text{G-6-P} \xrightleftharpoons{\text{G-6-PDH}} \text{H}^+ + \beta\text{-NADH} + \text{6-phosphate glucose lactone}$$

At that time the development of enzyme engineering didn't as good as today, the reagents only lied in two vials because the key technology did not be resolved. In case the reagents made into a single vial, the storage life was only between one to three months. In 1990's F Hoffman la Roche AG(AU-A-61906/90) had done much work based on Modrovich's principle. But his method can only prepare the double reagent, once prepared in the single reagent, the stability is poor. Klose et al (in US4,019,916) put forward a similar Method, but that took a long time to test and it was only suitable for testing system in which there is a substrate which can be phosphated. De Giorgio et al (Australia) in February 26,1996 applied their patent in China (CN1179792A). In that patent non-specific enzyme / substrate pair was successfully used in single reagent (AST, ALT) and two reagent (UREA), based on dynamic stabilization technology. The shelf life of the single liquid reagent (AST,ALT) was extended to 6-8 months. Although De Giorgio et al made improvement on predecessor's foundation, in the patent he claimed that the enzyme had incomplete specificity to the substrate and the enzyme / substrate pair was limited to glucose-6-phosphate dehydrogenase / D-glucose. The quantities of glucose-6-phosphate dehydrogenase / D-glucose are very big : glucose-6-phosphate dehydrogenase 3500U/L, D-glucose 18.016g/L. This not only obviously increases the cost, but also raises the possibility of introducing other enzymes in it.

Invention Description

In view of the existing technical insufficiency of the tests, in this invention we claime an enzymatic method for determination of analyte concentration in patient.

Said reagent relates to determine the oxidation rate of reduced coenzyme. It certainly not obviously increases the cost ,but can prevent other enzymes introduction, and has a long-term stability.

Said reagent is stabilized throughout storage by coenzyme reduction system of special enzyme/substrate pair in which coenzyme can be regenerated. Said enzyme is highly special for said substrate in the enzyme/substrate pair .

Said reagent is configured as a single vial in liquid;

glucose dehydrogenase/D-glucose pairs is prior to other enzyme/substrate pairs in coenzyme reduction system.

The invention also describes the enzyme reagent for determining the concentration of **aspartate aminotransferase.** The oxidation rate of reduced coenzyme is determined in the assay. Said reagent in a single vial is stabilized by the regeneration of reduction coenzyme at storage life based on coenzyme reduced system comprising special enzyme / substrate pair wherein said enzyme is special for said substrate. Glucose dehydrogenase/D-glucose pair is prior to other enzyme/substrate pairs. The concentration of said glucose dehydrogenase is in the range of 2-100U/L (5-50 U/L is optimal) and D-Glucose is in the range of 0.1-20mmol/L (1-10 mmol/L is optimal).

The invention also describes the enzyme reagent for determining the concentration of **alanine aminotransferase**. The oxidation rate of reduced coenzyme is determined at the test. Said reagent in a single vial is stabilized by the regeneration of reduced coenzyme at storage life based on coenzyme reduction system comprising special enzyme/substrate pair wherein said enzyme is special for said substrate. Glucose dehydrogenase/ D-glucose pair is prior to other enzyme/substrate pairs. The concentration of said glucose dehydrogenase is in the range of 2-100U/L (5-50 U/L is optimal) and D-glucose is in the range of 0.1-20mmol/L (1-10 mmol/L is optimal).

The invention also describes the enzyme reagent for determining the concentration of **urea**. The oxidation rate of reduced coenzyme is determined at the test. Said reagent in a single vial is stabilized by the regeneration of reduced coenzyme at storage life based on coenzyme reduction system comprising special enzyme/substrate pair wherein said enzyme is special for said substrate. Glucose dehydrogenase/ D-glucose pair is prior to other enzyme/substrate pairs. The concentration of said glucose dehydrogenase is in the range of 2-100U/L (5-50 U/L is optimal) and D-glucose is in the range of 0.1-20mmol/L (1-10 mmol/L is optimal).

In the regenration system ofβ-NADH comprising dehydrogenase/substrate pair, glucose dehydrogenase is completely special for D-glucose. D-glucose is converted to D- glucose lactone accompanying the reduction of β-NAD^+ to β-NADH. The reaction is as follows:

$$\beta\text{-NAD+ D-glucose} \xrightleftharpoons{\text{GDH}} \text{D- glucose lactone} + \beta\text{-NADH} + H^+$$

Glucose dehydrogenase is stable at pH 6-8.5 in test, so the reagent is stable in test at pH 7.2-8.5. However the optimum pH 8.0 of glucose dehydrogenase is in the range of pH 7.2-8.5. Because the enzyme is in this circumstance wherein enzyme reaction rate is fast and enzyme is in the prior pH, the quantity of dehydrogenase and substrate is

highly reduced. As a result, not only the reagent stability is improved because of avoiding contamination other enzymes but also the cost falls down.

The rate of regenerated β-NADH is controlled by modulating the quantity of glucose dehydrogenase and glucose in the reagent. In general, the rate of β-NADH regeneration is same as the rate of β-NADH oxidation. The coenzyme can be regenerated in coenzyme reduction system of regeneration, which has no effect on the assay.

In theβ-NADH regeneration systems the concentration of glucose dehydrogenase is in the range of 2-100U/L and glucose is in the range of 0.1-20mmol/L. Higher concentration of glucose dehydrogenase or glucose will result that the rate of regenerationβ-NADH is too fast. And the negative interference will come out in the assay.

As said in this invention that reagent used to determine AST in the sample comprises not only coenzyme reduction system including glucose dehydrogenase/ D-glucose but also malate dehydrogenase(MDH), lactate dehydrogenase, reduced coenzyme I(β-NADH), L- aspartate and 2-ketoglutarate .

Reagents in this invention used to detect ALT in the sample comprises not only glucose dehydrogenase/ D-glucose as coenzyme reduction system but also lactate dehydrogenase(LDH), reduced coenzyme I (β-NADH) and 2-ketoglutarate and so on.

Reagents in this invention used to determine the concentration of urea in the sample comprise not only glucose dehydrogenase/D-glucose as coenzyme reduced system but also urease, glutamate dehydrogenase (GLDH), reduced coenzyme I (β-NADH) and 2-ketoglutarate.

Reagents in the invention comprise not only essential coenzyme reduction system ,basic substrate and enzyme but also the buffer, preservative, stabilizer and chelator and so on, and more substances which can strengthen the stability but not affect the determination.

Glycerine, sugar and glycol are the polyhydroxylated compounds,which can form many hydrogen bonds with the protein molecules, which is helpful to the formation of 'solvent layer'. The solvent layer aroud the the enzyme molecules is different from the overall aqueous phase because it can improve the surface tension and solution viscosity. This kind chemical additive through effective dehydration can reduce the hydrolysis of the protein and therefore stabilize the enzyme. The enzyme can be stabilized by using the relatively low molecular weight polyols. We select glyceroland glycol as the stabilizers. But too high concentration is adverse to the detection because of the high solution viscosity.

EDTA disodium and heavy metal ions can form coordinate compound to avoid enzyme being inhibited by the heavy metal ion.

The microorganism pollution can reduce the stability of enzyme, addition of antiseptic may suppress the microorganism growth. In this invention, azide sodium is prior to other preservatives.

In this invention liquid reagents based on stabilization technology of coenzyme applying glucose dehydrogenase/ D-glucose pair are configured as a single

vial. And said reagents to determine AST mainly comprise coenzyme reduction system (glucose dehydrogenase/D-glucose), l-aspartate,α-oxoglutar-ate, malate dehydrogenase (MDH), lactate dehydrogenase, reduced coenzyme I (β-NADH).

In addition, prior reagents selected comprise Tris-HCl buffer, potassium hydroxide, EDTA disodium salt, glycerol, sodium azide.

The concentration of tris-HCl buffer is in the range of 20-100mmol/L; The concentration of α-oxoglutarate is limited in the range of 6-18 mmol/L because of it shows absorbency at 340nm; the concentration of L- aspartate is in the range of 100-300 mmol/L; The concentration of potassium hydroxide is the same as L-aspartate to increase the solubility of L-aspartate; The concentration of EDTA disodium is in the range of 1-10mmol/L in order to prevent the suppression of heavy metal ions to enzyme activity through formation coordinate compound with heavy metal ions; The concentration of β-NADH is in the range of 0.1-0.3mmol/L. When the concentration of β-NADH is lower than 0.1mmol/L the assay result is affected since the linear scope range being shortened.while the concentration of β-NADH is more than 0.3mmol/L the blank absorbency is too high for assay; The concentration of malate dehydrogenase (MDH) is in the range of 100-2500U/L; The addition of lactate dehydrogenase in the range of 1000-4000 U/L is to eliminate the interference from pyruvate in the sample ; The addition of glucose dehydrogenase/D-glucose in the range of 2-100U/L aims at the regeneration of β-NADH from it's oxidation product (NAD) so as to assure the stabilization of β-NADH; The concentration of D-glucose is in the range of 0.1-20mmol/L; The amount of glycerol is 1%-20% to make enzymes more stable. Higher concentration of glycerol will increase the viscosity of solution. The concentration of sodium azide is in the range of 0.1-1.0g/L to prevent the microorganism pollution.

According to the invention one kind of optimal reagent to determine AST comprises the ingredients being listed in the following table:

Stable 1

ingredients	Molecular weight	concentration (mmol/L)	/L
tris	121.1		
sodium α- oxoglutarate, ($2H_2O$)	226.1		
L- aspartate	133.1		
potassium hydroxide	56.1		
D-glucose	180.2		
glycerol	92.1		
EDTA.2Na	372.2		

sodium azide	65.1		
β-NADH disodium	709.4		
lactate dehydrogenase			
malate dehydrogenase			
glucose dehydrogenase			
hydrochloric acid	36.5		

In this invention liquid reagents based on stabilization technology of coenzyme applying glucosedehydrogenase/D-glucose are configured as a single vial. And said reagents to determine ALT mainly comprise coenzyme reduction system (glucose dehydrogenase/D-glucose), L-alanine, α-oxoglutarate, lactate dehydrogenase, reduced coenzyme I (β-NADH). In addition the prior reagents comprise Tris-HCl buffer, EDTA disodium, glycerol and sodium azide. The concentration of Tris-HCl buffer is prior in the range of 20-100mmol/L; The concentration of α-oxoglutarate is in the range of 8-18 mmol/L; The concentration of L-alanine is in the range of 200-800 mmol/L; The concentration of EDTA disodium is in the range of 1-10mmol/L; The concentration of β-NADH is in the range of 0.1-0.3mmol/L; lactate dehydrogenase added in the range of 1000-400 U/L is used to eliminate the interference from pyruvate in the sample and to assure catalyzing reaction in the linear range The concentration of glucose dehydrogenase is in the range of 2-100 U/L and D-glucose is 0.1-20mmol/L. The concentration of sodium azide is in the range of 0.1-100.0g/L. The concentration of glycerol is in the range of 1%-20%.

According to this invention one kind of optimal reagents to determine ALT comprises the ingredients being listed in the following table:

Stable 2

ingredients	Molecular weight	concentration (mmol/L)	Amount (g/L)
tris	121.1		
sodium α-ketoglutarate·$2H_2O$)	226.1		
L-alanine	89.1		
D-glucose	180.2		
EDTA·2Na	372.2		
glycerol	92.1		

sodium azide	65.1		
β-NADH disodium	709.4		
lactate dehydro-genase			
glucose dehydro-genase			
hydrochloric acid	36.5		

In this invention liquid reagents based on stabilization technology of coenzyme applying glucose dehydrogenase/ D-glucose are configured as a single vial. And said reagents to determine urea mainly comprise coenzyme reduction system (glucose dehydrogenase/ D-glucose), α-oxoglutarate, urease, glutamate dehydrogenase(GLDH), reduced coenzyme I(β-NADH).

In addition the optimal reagents comprise tris-HCl buffer, potassium adenosine diphosphate, glycerol and sodium azide. The concentration of Tris-HCl buffer is in the range of 20-150mmol/L; The concentration of α-oxoglutarate is in the range of 1-15mmol/L; The concentration of β-NADH is in the range of 0.10-0.38mmol/L; The concentration of potassium ADP is in the range of 0.1-10.0mmol/L; The concentration of urease is in the range of 2000-10000U/L for fast catalyzing decomposition of urea; Addition of glutamate dehydrogenase can control the reaction rate, more higher concentration will make the reaction rate quicker. The optimal concentration of glutamate dehydrogenase is in the range of 200-2000 U/L; The concentration of Glucose dehydrogenase is in the range of 2-100 U/L; The concentration of D- glucose is in the range of 0.1-20 mmol/L; The concentration of sodium azide is in the range of 0.1-1.0g/L; The concentration of glycerol is in the range of 1%-30%.

According to this invention one kind of optimal reagent to determine UREA includes ingredients being listed in the following table:

Stable 3

ingredients	Molecular weight	Concentration (mmol/L)	/L
tris	121.1		
α-ketoglutarate	226.1		
sodium azide	65.1		
D-glucose	180.2		

glycerol	92.1		
ADP.2K	501.3		
β-NADH disodium	709.4		
GLDH			
urease	709.4		
glucose dehydrogenase			
hydrochloric acid	36.5		

In the liquid single reagent, the selected Lactate dehydrogenase should have higher affinity with pyruvic acid, and contain micro absent from other enzyme such as ALT, GLDH and so on. The selected malic dehydrogenase and glutamic dehydrogenase should be more stable in aquesous solution. On the premise of keeping test linearity, delay time, accuracy and stability, the amount of the above enzymes should be reduced as far as possible, in order to eliminate the interference of other enzyme.

In addition to AST, ALT and UREA, the analytes which can be determined by the reagents of the invention include ammonia, lactate dehydrogenase, carbon dioxide, α-hydroxyl butyrate dehydrogenase and so on.

Furthermore, β-NADPH can be regenerated from NADP produced by oxidation in the case of glucose dehydrogenase/D-glucose.
The reaction is as follows:

$$\text{D-glucose} + \text{NADP}^+ \xrightleftharpoons{\text{GDH}} \text{D-gluconolactone} + \beta\text{-NADPH} + \text{H}^+$$

In contrast to the existing technology, the merit of this invention is as follows: the quantity of enzyme and substrate is reduced by the introduction of coenzyme reduction system against oxidation comprising highly specific enzyme/substrate pair.As a result, the other enzymes are excluded. So the cost of the reagent is reduced, but the stability is improved.

PREFERRED EMBODIMENTS

The details of this invention are clearly described by the following preferred examples.

Example 1

Determination of the stability of AST reagent (D-glucose: 5mmol/L, glucose dehydrogenase: 20U/L) formulated in accordance with the invention is as follows.

One kind of stabilized AST reagent in a single vial is as follows:

Stable 4

ingredients	Molecular Weight	Concentration (mmol/L)	/L

tris	121.1		
α-ketoglutarate, ($2H_2O$)	226.1		
L- aspartate	133.1		
potassium hydroxide	56.1		
D- glucose	180.2		
glycerol	92.1		
EDTA.2Na	372.2		
sodium azide	65.1		
β-NADH disodium	709.4		
lactate dehydrogenase			
malate dehydrogenase			
glucose dehydrogenase			
hydrochloric acid	36.5		

The correspondent unstabilized liquid reagent configured as a single vial does not include the coenzyme reduction system of Glucose dehydrogenase/D-glucose and absent from glycerol, but comprises the ingredients as above stable.

STORAGE CONDITIONS:

Sealed up and stored at 2-8℃ or 37℃

SPECTROPHOTOMETRIC PARAMETERS:

wavelength: 340nm reaction temperature: 37 ℃

cuvette path length: 10mm

volume ratio of sample to reagent: 1:15

lag phase: 60 seconds reaction time : 60 seconds

blank absorbance of reagent: > 1.0A (for showing the concentration of NADH)

assay precision: the result should be in the range of the quality control serum.

linearity range: ≥550U/L

assayed results:

1) The initial absorbance of AST reagent stored at 37 ℃

Table 5

storage at 37 ℃ (days)	Stabilized reagent	Unstabilized reagent
0	1.788	1.809
1	1.662	

2	1.538	
3	1.415	1.019
4	1.313	
5	1.188	
6	1.148	
7	1.043	

It is obvious that stabilized AST reagent in a single vial has a storage of seven days at 37 ℃. But the unstabilized AST reagent has only a storage of three days at 37 ℃. β-NADH in the stabilized reagent is more stable than others.

2) The initial absorbance of AST reagent stored at 2-8 ℃

Table 6

Storage at 2-8 ℃	Stabilized reagent	Unstabilized reagent
0 week	1.839	1.809
3 weeks		1.452
5 weeks		1.319
7 weeks		1.210
11 weeks		1.023
3 months	1.665	
6 months	1.477	
9 months	1.305	
12 months	1.102	

β-NADH in the stabilized AST reagent in a single has a storage more than 12 months at 2-8 ℃. Butβ-NADH in the unstabilized AST reagent has a storage of 11 weeks at 2-8 ℃.

3) linearity assay of stabilized AST reagent in a single vial stored at 2-8 ℃.

Table 7

stored at 2-8 ℃ for three months						
theory value U/L	0	116	233	349	466	582
assay value U/L	4.8	111	227	349	452	585
stored at 2-8 ℃ for six months						
theory value U/L	0	113	226	338	451	564
assay value U/L	5.1	115	220	338	436	559
stored at 2-8 ℃ for nine months						
theory value U/L	0	105	210	315	420	524
assay value U/L	4.9	106	215	315	406	517
stored at 2-8 ℃ for thirteen months						
theory value U/L	0	122	244	366	488	610
assay value U/L	5.5	124	247	366	473	582

The result of linearity assay of the stabilized AST reagent in a single vial is up to the mustard after being stored at 2-8 ℃ for thirteen months.

4) accuracy assay of stabilized AST reagent in a single vial stored at 37 ℃

Table 8

Storage at 37 ℃ (days)	Serum I (U/L) target value 30 (20-40)	Serum II (U/L) target value 54 (41-67)	Serum III (U/L) target value 101 (81-121)
0	28	54	98

5	28	56	92
6	27	52	95
7	26	53	95

The results of accuracy assay of the stabilized AST reagent in a single vial accord with the target values of the quality-controlled serum after being stored at 37 ℃ for seven days.

5) Accuracy assay of stabilized AST reagent stored at 2-8 ℃

Table 9

storage at 2-8℃ (months)	Serum I (U/L)	Serum III (U/L)
3	target value: 28(23-33) assay value:32	target value: 104(84-124) assay value: 117
6	target value: 28(23-33) assay value:32	target value: 104(84-124) assay value:101
9	target value: 28(23-33) assay value:30	target value: 104(84-124) assay value:110
12	target value: 30(20—40) assay value:32	target value: 101(81—121) assay value:106

The result of accuracy assay of the stabilized AST reagent in a single vial are all within the scope of the target value of the quality-controlled serum after being stored at 2-8 ℃ for 12 months.

The above results showed that the assay data of the stabilized AST reagent in a single vial was up to the mustard after being stored at 2-8 ℃ for twelve months or at 37 ℃ for seven days. In short, the method of utilizing coenzyme (NADH) reduced system of glucose dehydrogenase/D-glucose pair is feasible.

Example 2

Determination of the stability of AST reagent (D-glucose: 1mmol/L, glucose dehydrogenase: 5U/L) formulated in accordance with the invention is as follows.

One stabilized AST liquid reagent in a single vial is as follows:

Stable 10

ingredients	Molecular Weight	Concentration (mmol/L)	/L
Tris	121.1		
α- ketoglutarate ($2H_2O$)	226.1		
L- aspartate	133.1		
potassium hydroxide	56.1		
D- glucose	180.2		
glycerol	92.1		
EDTA.2Na	372.2		
sodium azide	65.1		
β-NADH disodium	709.4		
Lactate dehydrogenase			
malate dehydrogenase			
glucose dehydrogenase			
hydrochloric acid	36.5		

A correspondent unstabilized liquid reagent configured as a single vial does not include the coenzyme reduction system of glucose dehydrogenase/D-glucose and absent from glycerol, but comprises other ingredients as described in stable 10.

STORAGE CONDITIONS:

Seal up and stored at 2-8℃ or 37℃

SPECTROPHOTOMETRIC PARAMETERS:

wavelength: 340nm reaction temperature: 37 ℃

cuvette path length: 10mm
volume ratio of sample to reagent: 1:15
lag phase: 60 seconds reaction time : 60 seconds
bank absorbance of reagent: > 1.0A (for showing the concentration of NADH)
assay precision: the result should be in the range of the quality controlled serum.
linearity range: ≥550U/L
assay results:

2) The blank absorbance of AST liquid reagent stored at 37 ℃

Table 11

Storage at 37 ℃ (days)	Stabilized reagent	Unstabilized reagent
0	1.755	1.745
1	1.624	
2	1.490	
3	1.359	0.943
4	1.245	
5	1.110	

2) The blank absorbance of AST reagent in a single vial stored at 2-8 ℃

Table 12

Storage at 2-8 ℃	Stabilized reagent	Unstabilized reagent
0 week	1.758	1.751
3 weeks		1.396
5 weeks		1.258
7 weeks		1.150
11 weeks		0.965
3 months	1.579	
6 months	1.385	
9 months	1.208	
12 months	0.998	

3) linearity assay of stabilized AST reagent in a single vial stored at 2-8 ℃.

Table 13

stored at 2-8 ℃ for three months						
theory value U/L	31	125	250	374	499	624
assay value U/L	36	127	250	365	497	606
stored at 2-8 ℃ for six months						
theory value U/L	30	122	243	365	486	608
assay value U/L	33	122	258	365	483	578
stored at 2-8 ℃ for nine months						
theory value U/L	30	110	220	330	440	550
assay value U/L	35	118	229	325	436	538

4) accuracy assay of stabilized AST reagent in a single vial stored at 37 ℃

Table 14

storage at 37 ℃ (days)	Serum I (U/L) Target value 30 (20-40)	Serum II (U/L) Target value 54 (41-67)	Serum III (U/L) Target value 101 (81-121)
0	33	56	107
5	35	58	105

5) accuracy assay of stabilized AST reagent in a single vial stored at 2-8 ℃

Table 15

storage at 2-8℃ (months)	Serum I (U/L)	Serum III (U/L)

3	target value: 30(20-40) assay value:36	target value: 101(81-121) assay value: 115
6	Target value: 30(20-40) assay value:35	target value: 101(81-121) assay value:108
9	Target value: 30(20-40) assay value:32	target value: 101(81-121) assay value:106

The above results showed that the assay data of the stabilized AST reagent are up to the mustard after being stored at 2-8 ℃ for 9 months or at 37 ℃ for 5 days. In short, the method of utilizing coenzyme (NADH) reduction system of glucose dehydrogenase/D-glucose pair is feasible.

Example 3

Determination of the stability of AST reagent (D-glucose: 10mmol/L, glucose dehydrogenase: 50U/L) formulated in accordance with the invention is as follows.

One stabilized AST liquid reagent in a single vial is as follows:

Stable 16

ingredients	Molecular Weight	Concentration (mmol/L)	/L
tris	121.1		
Sodium α-oxoglutarate, ($2H_2O$)	226.1		
L- aspartate	133.1		
potassium hydroxide	56.1		
D- glucose	180.2		
glycerol	92.1		
EDTA.2Na	372.2		
sodium azide	65.1		

β-NADH disodium	709.4		
lactate dehydrogenase			
malate dehydrogenase			
glucose dehydrogenase			
hydrochloric acid	36.5		

The correspondence unstabilized liquid reagent configured as a single vial does not include the coenzyme reduction system of glucose dehydrogenase/D-glucose and absent from glycerol, but comprises the other ingredients as described in stable 16.

STORAGE CONDITIONS:

Sealed up and stored at 2-8℃ or 37℃

SPECTROPHOTOMETRIC PARAMETERS:

wavelength: 340nm reaction temperature: 37 ℃

cuvette path length: 10mm sample to reagent volume: 1:15

lag phase: 60 seconds reaction time : 60 seconds

initial absorbance of reagent: > 1.0A (to determine the concentration of NADH)

assay precision: assay result should be in the range of the quality control serum.

Linearity range: ≥550U/L

assay result:

3) The blank absorbance of AST liquid reagent in a single vial stored at 37 ℃

Table 17

Storage at 37 ℃ (days)	Stabilized reagent	Unstabilized reagent
0	1.880	1.886
1	1.759	
2	1.640	
3	1.518	1.075
4	1.396	
5	1.285	
6	1.177	
7	1.080	

2) The blank absorbance of AST liquid reagent stored at 2-8 ℃

Table 18

Storage at 2-8 ℃	Stabilized reagent	Unstabilized reagent
0 week	1.878	1.883
3 weeks		1.530
5 weeks		1.395
7 weeks		1.283
11 weeks		1.092
3 months	1.720	
6 months	1.541	
9 months	1.375	
12 months	1.192	

3) linearity assay of stabilized AST liquid reagent in a single vial stored at 2-8 ℃.

Table 19

stored at 2-8 ℃ for three months						
theory value U/L	0	121.7	244.1	366.0	488.0	600
assay value U/L	4.5	124	247	366	473	582
stored at 2-8 ℃ for six months						
theory value U/L	0	113	226	338	451	564
assay value U/L	5.0	115	220	338	436	559
stored at 2-8 ℃ for nine months						
theory value U/L	0	121	222	315	419	548
assay value U/L	4.6	115	220	315	420	535
stored at 2-8 ℃ for twelve months						
theory value U/L	0	114	228	343	457	571

assay value U/L	5.2	114	231	346	449	548

4) accuracy assay of stabilized AST liquid reagent in a single vial stored at 2-8 ℃

Table 20

storage at 2-8℃ (months)	Serum I (U/L)	Serum III (U/L)
3	target value: 28(23-33) assay value:31	target value: 104(84-124) assay value: 105
6	target value: 28(23-33) assay value:29	target value: 104(84-124) assay value: 96
9	target value: 28(23-33) assay value:29	target value: 104(84-124) assay value: 100
12	target value: 28(23-33) assay value:28	target value: 104(84-124) assay value: 105

5) accuracy assay of stabilized AST liquid reagent in a single vial stored at 37 ℃

Table 21

Storage at 37 ℃ (days)	Serum I (U/L) Target value 30 (20-40)	Serum II (U/L) Target value 54 (41-67)	Serum III (U/L) Target value 101 (81-121)
0	28	57	100
5	30	58	105
6	29	56	104

7	27	61	102

The above results showed that the assay data of the stabilized AST reagent was up to the mustard after being stored at 2-8 ℃ for 12 months or at 37 ℃ for 7 days. In short, the method of utilizing coenzyme (β-NADH) reduction system of glucose dehydrogenase/D-glucose pair is feasible.

Example 4

Determination of the stability of ALT reagent (D-glucose: 5mmol/L, glucose dehydrogenase: 10U/L) formulated in accordance with the invention is as follows.

One stabilized ALT reagent in a single vial is as follows:

Stable 22

Ingredients	Molecular Weight	Concentration (mmol/L)	/L
Tris	121.1		
α- ketoglutarate, ($2H_2O$)	226.1		
L- alanine	89.1		
D- glucose	180.2		
glycerol	92.1		
EDTA.2Na	372.2		
sodium azide	65.1		
β-NADH disodium	709.4		
lactate dehydrogenase			
glucose dehydrogenase			
hydrochloric acid	36.5		

The correspondence unstabilized liquid reagent configured as a single vial does not include the coenzyme reduction system of glucose dehydrogenase/D-glucose and absent from glycerol, but comprises the other ingredients as above stable 22.

STORAGE CONDITIONS:

Sealed and stored 2-8℃ or 37℃

SPECTROPHOTOMETRIC PARAMETERS:

wave length: 340nm reaction temperature: 37 ℃
cuvette path length: 10mm
volume ratio of sample to reagent: 1:15
lag phase: 60 seconds reaction time : 60 seconds
blank absorbance of reagent: > 1.0A for showing the concentration of NADH
assay accuracy: the result should be in the range of the quality controlled serum.
linearity range: ≥550U/L
assay result:
1) The blank absorbance of ALT liquid reagent in a single vial stored at 37 ℃
Table 23

Storage at 37 ℃ (days)	Stabilized reagent	Unstabilized reagent
0	1.857	1.752
1	1.687	1.375
2		1.066
3		
4	1.188	
5	1.057	

It is obvious that stabilized ALT reagent has a storage of 5 days at 37 ℃. But the unstabilized AST reagent has a storage of 2 days at 37 ℃. β-NADH in the stabilized reagent is more stable than others.

2) The blank absorbance of ALT liquid reagent in a single vial stored at 2-8 ℃
Table 24

Storage at 2-8 ℃	Stabilized reagent	Unstabilized reagent
0 week	1.902	1.752
3 months	1.688	1.235
4 months		1.041
6 months	1.468	
9 months	1.266	
12 months	1.099	

It is obvious that the storage of β-NADH in the stabilized ALT reagent is more

than 12 months at 2-8 ℃. Butβ-NADH in the unstabilized ALT reagent has a storage of 4 months only.

3) linearity assay of stabilized ALT liquid reagent in a single vial stored at 2-8 ℃.

Table 25

stored at 2-8 ℃ for three months					
Theory value U/L	4.3	153.4	302.5	451.5	600.6
assay value U/L	4.3	164.0	315.6	477.6	600.6
stored at 2-8 ℃ for six months					
theory value U/L	8.5	177.0	345.4	513.9	682.3
assay value U/L	8.5	199.1	367.9	541.4	682.3
stored at 2-8 ℃ for eight months					
theory value U/L	6.7	138.1	269.5	400.9	532.3
assay value U/L	6.7	157.8	293.2	416.5	532.3
stored at 2-8 ℃ for twelve months					
theory value U/L	5.9	154.1	302.3	450.5	598.7
assay value U/L	5.9	169.3	323.1	471.2	598.7

The results of linearity assay of the stabilized ALT liquid reagent in a single vial is up to the mustard after being stored at 2-8 ℃ for twelve months.

4) accuracy assay of stabilized ALT reagent stored at 37 ℃

Table 26

Storage at 37 ℃ (days)	Serum I (U/L) Target value 23 (18-28)	Serum II (U/L) Target value 45 (35-55)	Serum III (U/L) Target value 91 (76-106)

0	24.3	42.8	84.5
3	22.1	41.8	85.6
5	23.8	41.4	82.0

The result of accuracy assay of the stabilized ALT liquid reagent in a single vial are all within the target value scope of the quality-controlled serum after being stored at 37 ℃ for 5 days.

5) accuracy assay of stabilized ALT liquid reagent in a single vial stored at 2-8 ℃

Table 27

storage at 2-8℃ (months)	Serum I (U/L) target value: 24(19-29)	Serum III (U/L) target value: 92(77-107)
3	assay value:20	assay value: 82
6	assay value: 24	assay value: 78
9	assay value: 24	assay value: 84
12	assay value: 26	assay value: 88

The results of accuracy assay of the stabilized ALT liquid reagent in a single vial are all within the target value scope of the quality controlled serum after being stored at 2-8 ℃ for 12 months.

The above results showed that the assay data of the stabilized ALT reagent was up to the mustard after being stored at 2-8 ℃ for 12 months or at 37 ℃ for 5 days. In short, the method of utilizing coenzyme (NADH) reduction system of glucose dehydrogenase/D-glucose pair is sucessful.

Example 5

Determination of the stability of ALT reagent (D-glucose: 1mmol/L, glucose dehydrogenase: 2U/L) formulated in accordance with the invention is as follows.

One stabilized ALT liquid reagent in a single vial is as follows:

Stable 28

Ingredients	Molecular	Concentration	/L

	Weight	(mmol/L)	
tris	121.1		
α-ketoglutarate ($2H_2O$)	226.1		
L- alanine	89.1		
D- glucose	180.2		
glycerol	92.1		
EDTA.2Na	372.2		
sodium azide	65.1		
β-NADH disodium	709.4		
lactate dehydrogenase			
glucose dehydrogenase			
hydrochloric acid	36.5		

The correspondence unstabilized liquid reagent configured as a single vial does not include the coenzyme reduction system of glucose dehydrogenase/D-glucose and absent from glycerol, but comprises the ingredients as above stable.

STORAGE CONDITIONS:

Sealed up stored at 2-8℃ or 37℃

SPECTROPHOTOMETRIC PARAMETERS:

wavelength: 340nm reaction temperature: 37 ℃

cuvette path length: 10mm

volume ratio of sample to reagent 1:15

lag phase: 60 seconds reaction time : 60 seconds

bank absorbance of reagent: > 1.0A (for showing the concentration of NADH)

assay accuracy: the result should be in the range of the quality controlled serum.

Linearity range: ≥550U/L

assay results:

1) The blank absorbance of ALT reagent stored at 37 ℃

Table 29

Storage at 37 ℃ (days)	Stabilized reagent	Unstabilized reagent
0	1.710	1.705
1	1.525	1.330
2		1.019
3		

4	1.016	

2) The blank absorbance of ALT reagent stored at 2-8 ℃

Table 30

Storage at 2-8 ℃	Stabilized reagent	Unstabilized reagent
0 week	1.715	1.713
3 months	1.488	1.195
4 months		1.002
6 months	1.250	
9 months	1.031	

3) linearity assay of stabilized ALT liquid reagent in a single vial stored at 2-8 ℃.

Table 31

stored at 2-8 ℃ for three months						
theory value U/L	32	129	257	386	514	643
assay value U/L	30	128	257	376	507	629
stored at 2-8 ℃ for six months						
theory value U/L	3.9	104	208	312	417	520
assay value U/L	3.9	117	220	312	404	513
Stored at 2-8 ℃ for nine months						
theory value U/L	4.6	117	234	351	468	565
assay value U/L	4.6	112	237	351	459	536

4) accuracy assay of stabilized ALT reagent stored at 2-8 ℃

Table 32

storage at 2-8℃ (months)	Serum I (U/L) target value: 21(13-29)	Serum III (U/L) target value: 93(73-113)
3	assay value:28	assay value: 90
6	assay value: 24	assay value: 91
9	assay value: 22	assay value: 86

5) accuracy assay of stabilized ALT liquid reagent in a single stored at 37 ℃

Table 33

Storage at 37 ℃ (days)	Serum I (U/L) target value 24 (19-29)	Serum II (U/L) target value 47 (37-57)	Serum III (U/L) target value 92 (77-107)
0	22	51	90
4	28	46	87

The above result showed that the assay data of he stabilized ALT reagent was up to the mustard after being stored at 2-8 ℃ for 9 months or at 37 ℃ for 4 days. The method of utilizing coenzyme (NADH) reduction system of glucose dehydrogenase/D-glucose pair is sucessful.

Example 6

Determination of the stability of ALT reagent (D-Glucose: 10mmol/L, Glucose dehydrogenase: 50U/L) formulated in accordance with the invention is as follows.

One stabilized ALT liquid reagent in a single vial is as follows:

Stable 34

ingredients	Molecular Weight	Concentration (mmol/L)	/L
Tris	121.1		
α-ketoglutarate ($2H_2O$)	226.1		
L- alanine	89.1		
D- glucose	180.2		

glycerol	92.1		
EDTA.2Na	372.2		
sodium azide	65.1		
β-NADH disodium	709.4		
lactate dehydrogenase			
glucose dehydrogenase			
hydrochloric acid	36.5		

The correspondent unstabilized liquid reagent configured as a single vial does not include the coenzyme reduction system of glucose dehydrogenase/D-glucose and absent from glycerol, but comprises the ingredients as above stable34.

STORAGE CONDITIONS:

Sealed up and stored 2-8℃ or 37℃

SPECTROPHOTOMETRIC PARAMETERS:

wavelength: 340nm reaction temperature: 37 ℃

cuvette path length: 10mm

volume ratio of sample to reagent: 1:15

lag phase: 60 seconds reaction time : 60 seconds

blank absorbance of reagent: > 1.0A (for showing the concentration ofβ-NADH)

assay accuracy: assay result should be in the range of the quality controlled serum.

Linearity range: ≥550U/L

assay results:

1) The blank absorbance of ALT reagent stored at 37 ℃

Table 35

Storage at 37 ℃ (days)	Stabilized reagent	Unstabilized reagent
0	1.881	1.875
1	1.715	1.493
2		1.154
3		
4	1.220	
5	1.092	

2) The blank absorbance of ALT reagent stored at 2-8 ℃

Table 36

Storage at 2-8 ℃	Stabilized	Unstabilized reagent

	reagent	
0 week	1.915	1.880
3 months	1.697	1.365
4 months		1.169
6 months	1.478	
9 months	1.280	
12 months	1.115	

3) linearity assay of stabilized ALT liquid reagent in a single vial stored at 2-8 ℃.

Table 37

stored at 2-8 ℃ for three months						
Theory value U/L	5.1	142	284	425	567	
assay value U/L	5.1	142	294	412	555	
stored at 2-8 ℃ for six months						
theory value U/L	4.5	125	246	369	492	615
assay value U/L	4.5	121	244	372	487	604
Stored at 2-8 ℃ for nine months						
theory value U/L	5.5	80	160	321	481	641
assay value U/L	5.5	85	170	321	473	625
Stored at 2-8 ℃ for 12 months						
theory value U/L	98	195	293	390	488	580
assay value U/L	100	199	287	398	479	561

4) accuracy assay of stabilized ALT liquid reagent in a single vial stored at 2-8 ℃

Table 38

storage at 2-8℃ (months)	Serum I (U/L) target value: 21(13-29)	Serum III (U/L) target value: 93(73-113)
3	assay value:23	assay value: 84
6	assay value: 21	assay value: 86
9	assay value: 26	assay value: 82
12	assay value: 22	assay value: 87

5) accuracy assay of stabilized ALT liquid reagent in a single vial stored at 37 ℃

Table 39

Storage at 37 ℃ (days)	Serum I (U/L) Target value 24 (19-29)	Serum II (U/L) Target value 47 (37-57)	Serum III (U/L) Target value 92 (77-107)
0	21	45	86
5	23	44	84

The above result showed that the assay data of the stabilized ALT reagent was up to the mustard after being stored at 2-8 ℃ for 12 months or at 37 ℃ for 5 days. The method of utilizing coenzyme (β-NADH) reduction system of glucose dehydrogenase/D-glucose pair is sucessful.

Example 7

Determination of the stability of UREA reagent (D-glucose: 5mmol/L,glucose dehydrogenase: 30U/L) formulated in accordance with the invention is as follows.

One kind of stabilized UREA reagent in a single vial is as follows:

Stable 40

ingredients	Molecular Weight	Concentration (mmol/L)	/L
tris	121.1		
α- ketoglutarate ($2H_2O$)	226.1		
D- glucose	180.2		

glycerol	92.1		
ADP.K salt	501.3		
sodium azide	65.1		
β-NADH disodium	709.4		
glutamate dehydrogenase			
urease			
glucose dehydrogenase			
hydrochloric acid	36.5		

The correspondent unstabilized liquid reagent configured as a single vial does not include the coenzyme reduction system of glucose dehydrogenase/D-glucose pair and absent from glycerol, but comprises the ingredients as above stable 40.

STORAGE CONDITIONS:

sealed and stored at 2-8℃ or 37℃

SPECTROPHOTOMETRIC PARAMETERS:

wavelength: 340nm reaction temperature: 37 ℃

cuvette path length: 10mm

volume ratio of sample to reagent 1:100

lag phase: 30 seconds

reaction time : 60-150 seconds

blank absorbance of reagent: > 1.0A (for showing the concentration of NADH)

assay precision: assay result should be in the range of the quality controlled serum.

linearity range: ≥50mmol/L

assay result:

1) The blank absorbance of UREA reagent stored at 37 ℃

Table 41

Storage at 37 ℃ (days)	Stabilized reagent	Unstabilized reagent
0	1.821	1.835
1	1.655	1.621
2	1.547	1.424
3	1.421	1.267
4	1.302	1.113
5	1.200	0.956
6	1.126	

7	1.051	

It is obvious that stabilized UREA reagent has a storage of seven days at 37 ℃. But the unstabilized UREA reagent has a storage of four days at 37 ℃. β-NADH in the stabilized reagent is more stable

2) The initial absorbance of UREA reagent stored at 2-8 ℃

Table 42

Storage at 2-8 ℃ (months)	Stabilized reagent	Unstabilized reagent
0	1.773	1.835
3	1.611	1.519
6	1.507	1.271
8		1.035
9	1.391	
12	1.226	
15	1.125	
18	1.029	

The storage of β-NADH in the stabilized UREA reagent is more than 12 months at 2-8 ℃. But the storage of UREA reagent in the unstabilized is only 8 months at 2-8 ℃.

3) linearity assay of stabilized UREA reagent stored at 2-8 ℃.

Table 43

stored at 2-8 ℃ for four months						
theory value U/L	1.59	10.58	21.16	31.74	42.32	52.90
assay value U/L	1.92	11.18	22.21	31.74	43.69	52.89
stored at 2-8 ℃ for six months						

theory value U/L	1.68	14.00	28.00	42.00	56.00
assay value U/L	1.82	14.32	28.09	41.95	54.26
stored at 2-8 ℃ for nine months					
theory value U/L	1.62	13.50	27.00	40.50	54.00
assay value U/L	1.80	14.14	27.55	40.16	52.08
stored at 2-8 ℃ for twelve months					
theory value U/L	1.62	13.50	27.00	40.50	54.00
assay value U/L	1.74	14.07	27.86	39.67	51.70
stored at 2-8 ℃ for fifteen months					
theory value U/L	1.62	13.50	27.00	40.50	54.00
assay value U/L	1.80	13.39	27.00	38.12	50.15
stored at 2-8 ℃ for eighteen months					
theory value U/L	1.68	14.00	28.00	42.00	56.00
assay value U/L	1.80	14.59	28.44	40.61	53.38

The result of linearity assay of the stabilized UREA liquid reagent in a single vial is up to the mustard after being stored at 2-8 ℃ for eighteen months.

4) accuracy assay of stabilized UREA reagent stored at 37 ℃

Table 44

Storage at 37 ℃ (days)	Serum I (U/L) Target value 3.0 (2.3-3.7)	Serum II (U/L) Target value 10.2(8.7-11.7)	Serum III (U/L) Target value 18.7(16.5-20.8)
0	3.10	10.40	18.80
5	3.12	10.08	19.54
7	2.97	10.11	18.77

The results of accuracy assay of the stabilized UREA reagent within the scope

of target value of the quality-controlled serum after being stored at 37 ℃ for seven days.

5) accuracy assay of stabilized UREA reagent stored at 2-8 ℃

Table 45

storage at 2-8℃ (months)	Serum I (U/L)	Serum III (U/L)
3	target value: 2.5(1.8-3.2) assay value:2.76	target value: 18.5(16.3-20.7) assay valu: 18.06
6	Target value: 2.5(1.8-3.2) assay value:2.52	target value: 18.5(16.3-20.7) assay value:19.40
9	Target value: 2.5(1.8-3.2) assay value:2.72	target value: 18.5(16.3-20.7) assay value:19.05
12	Target value: 7.70(5.58—9.55) assay value:7.86	
15	Target value: 3.0(2.3—3.7) assay value:2.83	Target value: 18.7(16.5-20.8) Assay value:17.27
18	Target value: 3.0(2.3—3.7) assay value:2.95	Target value: 18.7(16.5-20.8) Assay value:19.05

The results of accuracy assay of the stabilized UREA liquid reagent within the scope of target value of the quality-controlled serum after being stored at 2-8 ℃

for 12 months.

The above results showed that the assay data of the stabilized UREA reagent was up to the mustard after being stored at 2-8 . for eighteen months or at 37 . for seven days. In short, the method of utilizing coenzyme (NADH) reduced system of glucose dehydrogenase/D-glucose pair is feasible.

Example 8

Determination of the stability of UREA reagent (D-Glucose: 1mmol/L, Glucose dehydrogenase: 5U/L) formulated in accordance with the invention is as follows.

One stabilized UREA reagent in a single vial is as follows:

Stable 46

ingredients	Molecular Weight	Concentration (mmol/L)	/L
Tris	121.1		
α-ketoglutarate ($2H_2O$)	226.1		
D- glucose	180.2		
glycerol	92.1		
ADP.K salt	501.3		
sodium azide	65.1		
β-NADH disodium	709.4		
glutamate dehydrogenase			
urease			
glucose dehydrogenase			
hydrochloric acid	36.5		

The correspondent unstabilized liquid reagent configured as a single vial do not include the coenzyme reduction system of glucose dehydrogenase/D-glucose and absent from glycerol, but comprises the ingredients as above stable 46.

STORAGE CONDITIONS:

sealed and stored at 2-8 or 37

SPECTROPHOTOMETRIC PARAMETERS:

wavelength: 340nm reaction temperature: 37

cuvette path length: 10mm
volume ratio of sample to reagent: 1:100
lag phase: 30 seconds reaction time : 60-150 seconds
blank absorbance of reagent: > 1.0A (for showing the concentration of NADH)
assay precision: assay results should be in the range of the quality controlled serum.
linearity range: ≥50mmol/L
assayed results:

2) The blank absorbance of UREA liquid reagent in a single vial stored at 37

Table 47

Storage at 37 (days)	Stabilized reagent	Unstabilized reagent
0	1.550	1.557
1	1.379	1.345
2	1.265	1.114
3	1.130	0.986
4	1.008	

2) The blank absorbance of UREA liquid reagent in a single vial stored at 2-8

Table 48

Storage at 2-8 (months)	Stabilized reagent	Unstabilized reagent
0	1.562	1.555
3	1.393	1.240
6	1.285	0.996
9	1.163	
12	1.001	

3) linearity assay of stabilized UREA reagent stored at 2-8 .

Table 49

stored at 2-8 for three months					
theory value U/L	1.70	12.80	25.60	38.40	51.20
assay value U/L	1.80	13.39	27.00	38.12	50.15
stored at 2-8 for six months					

theory value U/L	10.50	21.00	31.50	42.00	52.50
assay value U/L	11.36	22.11	31.75	41.61	52.69
stored at 2-8 ° for nine months					
theory value U/L	10.50	21.00	31.50	42.00	52.50
assay value U/L	11.12	21.84	31.61	41.50	50.18
stored at 2-8 for twelve months					
theory value U/L	10.22	20.44	30.66	40.88	51.10
assay value U/L	10.80	20.50	29.77	39.14	48.75

4) accuracy assay of stabilized UREA reagent stored at 37

Table 50

Storage at 37 (days)	Serum I (U/L) Target value 2.5(1.8-3.2)	Serum II (U/L) Target value 9.0(7.5-10.5)	Serum III (U/L) Target value 18.5(16.3-20.7)
0	2.67	9.83	18.14
4	2.85	9.77	18.50

5) accuracy assay of stabilized UREA reagent stored at 2-8

Table 51

storage at 2-8 (months)	Serum I (U/L) target value: 2.7(2.0-3.3)	Serum III (U/L) target value: 18.7(16.5-20.8)
3	2.91	19.20
6	3.00	19.01
9	2.98	18.90
12	2.90	19.00

The above results showed that the assay data of the stabilized UREA reagent

was up to the mustard after being stored at 2-8 for twelve months or at 37 for four days. In short, the method of utilizing coenzyme (NADH) reduction system of glucose dehydrogenase/D-glucose pair is feasible.

Example 9

Determination of the stability of UREA reagent (D-glucose: 10mmol/L, glucose dehydrogenase: 50U/L) formulated in accordance with the invention is as follows.

One kind of stabilized UREA reagent in a single vial is as follows:

Stable 52

ingredients	Molecular Weight	Concentration (mmol/L)	/L
tris	121.1		
α-ketoglutarate ($2H_2O$)	226.1		
D- glucose	180.2		
glycerol	92.1		
ADP.K salt	501.3		
sodium azide	65.1		
β-NADH disodium	709.4		
glutamate dehydrogenase			
urease			
glucose dehydrogenase			
hydrochloric acid	36.5		

The correspondent unstabilized liquid reagent configured as a single vial does not include the coenzyme reduced system of glucose dehydrogenase/D-glucose and absent from glycerol, but comprises the ingredients as above stable.

STORAGE CONDITIONS:

sealeded and stored at 2-8 or 37

SPECTROPHOTOMETRIC PARAMETERS:

wavelength: 340nm reaction temperature: 37

cuvette path length: 10mm

volume ratio of sample to reagent: 1:100

lag phase: 30 seconds reaction time : 60-150 seconds

blank absorbance of reagent: > 1.0A (for showing the concentration of NADH)

assay precision: assay results should be in the range of the quality controlled serum.
Linearity range: ≥50mmol/L
assay results:
3) The blank absorbance of UREA reagent stored at 37 .
Table 53

Storage at 37 (days)	Stabilized reagent	Unstabilized reagent
0	1.942	1.938
1	1.780	1.723
2	1.675	1.520
3	1.553	1.362
4	1.440	1.205
5	1.341	1.047
6	1.273	
7	1.205	

2) The blank absorbance of UREA liquid reagent in a single vial stored at 2-8
Table 54

Storage at 2-8 (months)	Stabilized reagent	Unstabilized reagent
0	1.933	1.930
3	1.770	1.613
6	1.669	1.362
8		1.124
9	1.555	
12	1.392	
15	1.295	
18	1.203	

3) Linearity assay of stabilized UREA reagent stored at 2-8 .

Table 55

stored at 2-8 for three months

theory value U/L	11.47	22.93	34.40	45.87	57.34
assay value U/L	11.96	23.02	34.40	43.62	55.43
stored at 2-8 for six months					
theory value U/L	10.03	20.06	30.09	40.12	50.15
assay value U/L	10.03	20.80	31.43	40.12	50.29
stored at 2-8 for nine months					
theory value U/L	11.30	22.60	34.00	45.30	56.60
assay value U/L	11.90	23.20	34.00	43.70	54.20
stored at 2-8 for twelve months					
theory value U/L	11.66	23.31	34.97	46.63	58.29
assay value U/L	12.07	24.44	34.97	46.22	56.96
stored at 2-8 for fifteen months					
theory value U/L	11.00	21.90	32.90	43.80	54.80
assay value U/L	11.80	22.40	31.70	43.80	52.30
stored at 2-8 for eighteen months					
theory value U/L	10.27	20.54	30.80	41.07	51.34
assay value U/L	11.09	21.73	31.95	41.07	50.81

4) The accuracy assay of stabilized UREA reagent stored at 37

Table 56

Storage at 37 (days)	Serum I (U/L) target value 2.5(1.8-3.2)	Serum II (U/L) target value 9.0(7.5-10.5)	Serum III(U/L)target value 18.5(16.3-20.7)
0	2.64	9.17	18.64
4	2.51	9.70	18.73
7	2.47	9.64	19.20

5) Accuracy assay of stabilized UREA liquid reagent stored at 2-8

Table 57

storage at 2-8℃ (months)	Serum I (U/L) target value: 2.7(2.0-3.3)	Serum III (U/L) target value: 18.7(16.5-20.8)
3	2.77	18.35
6	2.80	18.86
9	2.90	18.65
12	2.92	18.60
15	2.98	19.00
18	3.00	18.73

The above result showed that the assay data of the stabilized UREA reagent were up to the mustard after being stored at 2-8℃ for eighteen months or at 37℃ for seven days. In short, the method of utilizing coenzyme (NADH) reduced system of glucose dehydrogenase/D-glucose pair is feasible.

Industry usability

In this invention, because of utilization of an antioxidant coenzyme reduction system which comprises highly specific enzyme/substrate pair,so the amounts of enzyme and substrate are reduced greatly, and the cost of reagent does not increase almost. Moreover, the stability of the reagent is enhanced, which resulted from avoiding introduction of other enzymes along with the massive stable enzymes addition.